Mutual Feedbacks Maintain Both Genetic and Species Diversity in a Plant Community

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The forces that maintain genetic diversity among individuals and diversity among species are usually studied separately. Nevertheless, diversity at one of these levels may depend on the diversity at the other. We have combined observations of natural populations, quantitative genetics, and field experiments to show that genetic variation in the concentration of an allelopathic secondary compound in *Brassica nigra* is necessary for the coexistence of *B. nigra* and its competitor species. In addition, the diversity of competing species was required for the maintenance of genetic variation in the trait within *B. nigra*. Thus, conservation of species diversity may also necessitate maintenance of the processes that sustain the genetic diversity of each individual species.

A wealth of theoretical and empirical research has sought the mechanisms that enable multiple competing species to coexist in a given area (1–3). Similarly, much research in population and quantitative genetics explores processes that promote the maintenance of allelic variation (i.e., genetic diversity) at loci, especially for traits with potential selective impacts (4–6). Recent theoretical and empirical studies suggest that diversity at one level may depend on the diversity of the other (7–11). Selection pressure on specific traits varies with community composition, suggesting a role for species diversity in maintaining genetic diversity (12). Additionally, genetic diversity in plant populations can determine the species diversity of associated arthropods and microbes (7–9). However, no studies have explicitly studied how species and genetic diversity may interact, creating feedback loops that simultaneously maintain diversity at each level.

Plants in the family Brassicaceae all produce glucosinolates (amino acid–derived secondary compounds that consist of a β-thioglucose residue, an N-hydroxyiminosulfate moiety, and a variable side chain) that, in the presence of the myrosinase enzyme, break down into isothiocyanates and other products toxic to many herbivores, bacteria, mycorrhizal fungi, and other plants (13–15). Thus, glucosinolates affect a host of organisms and are ecologically important compounds in natural communities. We tested the hypothesis that changes in the mean level of a glucosinolate allochemical could lead to changes in plant community structure (potentially through allelopathic or antimycorrhizal effects) that, in turn, would feed back to affect selection on the allochemical. *Brassica nigra* was selected for study because *B. nigra*, unlike most other species of the Brassicaceae, has the desirable property that 90 to 99% of its glucosinolates are in the form of a single compound: sinigrin (allylglucosinolate), which is a heritable trait (16, 17). Because of sinigrin’s effects on heterospecific plants and mycorrhizal fungi (mutualists that benefit most plant species but not members of the Brassicaceae), we hypothesized that investment to sinigrin should benefit *B. nigra* genotypes competing with heterospecifics but not conspecifics.

To determine whether there were possible feedbacks between natural variation in community composition and variation in *B. nigra* sinigrin concentration, we sampled naturally occurring *B. nigra* individuals along a gradient ranging from a monospecific stand of pure *B. nigra* to areas dominated by a mix of other species (heterospecifics), with only a few widely spaced *B. nigra* individuals. We estimated the percentage of cover of four functional groups (*B. nigra*, heterospecific forbs, grasses, and bare ground) in a 1-m-diameter circle around each selected individual. We also measured the sinigrin concentration in leaves and final fitness for each individual (18).

We found that the selective value of sinigrin increased as the community became dominated by heterospecifics (Fig. 1). However, for *B. nigra* individuals growing mostly with conspecifics, high sinigrin concentrations were correlated with lower fitness (Fig. 1), resulting in a significant interaction between the effects of sinigrin concentration and percentage of cover of *B. nigra* on the fitness of individual plants (F1,43 = 6.065, *P* < 0.05, *R* 2 = 0.278, multiple linear regression). Additionally, the cover of heterospecific forbs immediately surrounding the sampled *B. nigra* individuals declined significantly with increasing sinigrin concentrations in the sampled *B. nigra* plants (Fig. 2). Thus, not only does the selective value of sinigrin concentration seem to depend on community composition, but the community composition may also depend on the sinigrin concentrations of individual plants.

Our observations suggest that cyclic dynamics between selection pressures and community composition could lead to the simultaneous maintenance of both genetic diversity in sinigrin genes and species diversity in the plant community. To test this hypothesis rigorously, we performed a community invasibility experiment. Using plants from six naturally occurring populations and three generations of artificial selection, we created lines of high- and low-sinigrin *B. nigra*; the mean values of sinigrin in both lines were within the natural range found in these populations (18).

For community diversity, we collected seeds from three abundant, co-occurring, and phylogenetically diverse competitor species [*Ansinekia menziesii* (Boraginaceae), *Sonchus oleraceus*...
B. nigra grows naturally both in dense monospecific stands and in diverse heterospecific stands with these three species in the field (Fig. 2). We made three replicated community types in the field: high-sinigrin B. nigra monocultures, low-sinigrin B. nigra monocultures, and a three-species mixed heterospecific community. Each community consisted of 24 neighbor plants surrounding one target invader. Into each synthetic community, we introduced one focal plant (as an “invader”). Invader plants were either a high- or low-sinigrin B. nigra or a heterospecific individual from one of the three other species. Plants were grown 25 cm apart, with 3-m alleys between communities. For each community type, we grew 11 communities with a high-sinigrin invader, 11 with a low-sinigrin invader, and 7 with each heterospecific species as the invader (18).

Thus, sinigrin concentrations and community composition (hetero- or conspecific) vary among our experimental communities, resulting in genetic and species diversity at the field rather than the plot scale. Theory has shown that competitive coexistence of species or alleles occurs only when all species (or genotypes) can invade when rare (19). Thus, this design allows us to test directly whether the genetic variation in sinigrin among our experimental communities will lead to the maintenance of species diversity (and vice versa) by testing whether all species and genotypes can invade at least one community type other than their own.

Consistent with observational data in the field, we found that high-sinigrin B. nigra invaders had greater fitness than low-sinigrin B. nigra genotypes when invading diverse heterospecific communities. In contrast, low-sinigrin genotypes were better than high-sinigrin genotypes at invading monospecific stands of B. nigra, regardless of the sinigrin level of the B. nigra community [invader genotype (high versus low sinigrin) by community (Brassica versus heterospecific) interaction; log-likelihood ratio (LR) = 4.10, P < 0.05] (Fig. 3, generalized linear model). Thus, the fitness of a B. nigra genotype depends on the community composition (diverse versus monospecific) that it invades.

Heterospecific invaders were sensitive to the genotype of B. nigra and had significantly lower fitness in communities of high-sinigrin versus low-sinigrin B. nigra genotypes [community (high versus low), LR = 6.54, P = 0.01] (Fig. 3). Despite low sample sizes, two of the three heterospecific species (A. menziesii and S. oleraceus) showed significant reductions when analyzed alone (LR = 4.05, P < 0.05 and LR = 5.41, P < 0.05 respectively), whereas the third (M. parviflora) showed a nonsignificant trend in the same direction.

Coexistence generally requires that species compete more strongly intra- versus interspecifically (1–3). Therefore, we also measured the fitness of these three competitor species when invading monospecific communities of their own species, to provide an estimate of pure intraspecific competition for each species. Interestingly, there was no difference in heterospecific invader fitness when invading a monoculture of their own species versus when invading an “average” B. nigra monoculture (combining high- and low-sinigrin communities) for any of the three heterospecific species (LR < 0.65, P > 0.40 for all). This result suggests that genetic variation among B. nigra competitors was more important than differences between competitor species in determining fitness of heterospecific competitors. Thus, if one ignores genetic variation in B. nigra, the requirement for coexistence does not seem to be met (for all three species, intraspecific competition is equal to or weaker than interspecific competition with “average” B. nigra). Only when one considers the role of genetic variation in the competitive ability of different B. nigra lines is coexistence predicted.

The observed differences in heterospecific invader fitness in seed production persisted into the next generation in the field, resulting in higher seedling densities in low-sinigrin communities compared with high-sinigrin communities (all species combined, LR = 5.06, P < 0.05; A. menziesii, LR = 4.59, P < 0.05; S. oleraceus, LR = 4.24, P < 0.05; M. parviflora, LR = 0.433, P = 0.51) (Fig. 4). Thus, genetic variation in B. nigra affected the population dynamics of competing species.

Because our analyses are based on the comparison of our artificially selected lines, it is possible that any effects we detect are due to pleiotropic effects or genes closely linked to those controlling sinigrin concentrations, rather than the direct action of sinigrin. The evolutionary implications are the same in either case, because our invasion analysis still predicts how sinigrin concentrations are expected to increase or decrease through time, whether through the direct action of sinigrin or as correlated responses to selection on closely linked genes. Nevertheless, we have some evidence that sinigrin itself may be driving these patterns. Glucosinolates have been extensively studied as defenses against herbivores (11, 12); however, rates of herbivory by generalist and specialist herbivores were generally low and did not differ between community or invader types.

Another possibility is that mycorrhizal fungal communities are altered by sinigrin or its breakdown products. Glucosinolates have known antifungal properties (13) and have been proposed to mediate competition between mustards and other plants through their effects on arbuscular mycorrhizal fungal mutualists of competitors (20). To explore this possibility, we estimated the mycorrhizal infection potential (MIP) of soils taken from 10 plots of each community type (high- and low-sinigrin B. nigra monocultures and mixed communities of the three heterospecifics), using Sorghum bicolor as an indicator species (18, 21).

The plant community type had a large, significant effect on the MIP of the soil samples (R² = 0.677, F₁,27 = 15.717, P < 0.001, analysis of variance). Soils from under high-sinigrin B. nigra communities had significantly reduced MIP compared with soils from low-sinigrin communities, with soils from diverse heterospecific communities intermediate between them (Fig. 5).

Fig. 3. Fitness of invaders in the three community types (means ± SE). Gray bars represent the average response of the three heterospecific species. See text for statistical comparisons.

Fig. 4. Seedling density of the three heterospecifics in the following growing season, comparing high- and low-sinigrin B. nigra communities into which a heterospecific “invader” of the same species had been planted in the previous year (means ± SE). Asterisks indicate significant (P < 0.05) differences between high- and low-sinigrin communities within an invading species.
quantitative imaging of Nitrogen Fixation by Individual Bacteria Within Animal Cells

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Biological nitrogen fixation, the conversion of atmospheric nitrogen to ammonia for biosynthesis, is exclusively performed by a few bacteria and archaea. Despite the essential importance of biological nitrogen fixation, it has been impossible to quantify the incorporation of nitrogen by individual bacteria or to map the fate of fixed nitrogen in host cells. In this study, with multi-isotope imaging mass spectrometry we directly imaged and measured nitrogen fixation by individual bacteria within eukaryotic host cells and demonstrated that fixed nitrogen is used for host metabolism. This approach introduces a powerful way to study microbes and global nutrient cycles.

Bacteria and archaea responsible for biological nitrogen fixation can be found in free-living form (1–3) or in symbiosis with algae (4, 5), higher plants (3, 5), and some animals (6–8). Although these microbes are a critical part of the global nitrogen cycle (9), there has previously been no means to evaluate this fixation process at subcellular resolution. This is now possible with multi-isotope imaging mass spectrometry (MIMS) (10).

Wood and woody plant materials are abundant in the biosphere (11) and are important nutrient sources for a variety of fungi and microorganisms (12). Yet few animals are able to feed primarily on wood (13). Although rich in carbon, wood typically contains two orders of

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Plants in the Brassicaceae are characterized by low mycorrhizal potential, whereas we observed that the three heterospecific competitors, like almost all other angiosperms, do form mutualistic associations with mycorrhizal fungi. Therefore, the observed pattern in MIP among community types may explain the pattern of lower fitness of heterospecifics in high-sinigrin B. nigra communities and higher fitness of high-sinigrin B. nigra invaders in heterospecific communities. The higher fitness of low-sinigrin B. nigra invaders in B. nigra monocultures is consistent with a cost of sinigrin production (22) and little benefit of high sinigrin levels when competing with non-mycorrhizal B. nigra neighbors. Other mechanisms, such as direct allelopathy, may be acting as well (23).

Our field data show an intransitive competitive hierarchy between competing species and genotypes leading to cyclical dynamics; high-sinigrin B. nigra can invade diverse communities of other species, low-sinigrin B. nigra can invade patches of high-sinigrin B. nigra, and other species can invade patches of low-sinigrin B. nigra. Thus, each species or genotype is able to invade at least one other community type, promoting coexistence through mutual invasibility (19). For instance, a diverse, heterospecific community could be invaded by high-sinigrin genotypes of B. nigra. As B. nigra rises in abundance, displacing heterospecifics, selection will begin to favor lower-sinigrin concentrations. If sinigrin concentrations fall low enough, heterospecific species may be able to reinvade the community, starting the cycle over. This kind of “rock-paper-scissors” intransitivity has been shown to allow coexistence between species (24, 25) and genotypes within species (26, 27), but few studies have investigated intransitive networks consisting of different species and genotypes within one species (28).

The experimental results, combined with natural observations, show that in this system, the maintenance of species diversity is dependent on sufficient genetic variation, because without this variation the system would become dominated by B. nigra (if sinigrin levels are uniformly high), or by other species (if sinigrin levels are uniformly low). Simultaneously, the maintenance of genetic variation is dependent on species diversity, because selection is predicted to fix sinigrin levels at their lowest level if other competing species are not present. Our experiments show that a trade-off between intra- and interspecific competitive ability in the genetically variable species led to an intransitive competitive hierarchy among competing species and genotypes, thereby promoting coexistence. These results clearly show the potential for genetic variability and microevolution within species to alter community dynamics and structure. Conservation efforts aimed at maintaining species diversity therefore should not overlook the potential impacts of losses of genetic diversity, which could ultimately lead to losses of interacting species.

References and Notes